IDSA 2010
214

Antimicrobial Activity of Ceftaroline Combined With NXL104 When Tested Against Enterobacteriaceae Producing Derepressed AmpC β-lactamase

H.S. SADER, D.J. FARRELL, M. CASTANHEIRA, R.N. JONES
JMI Laboratories, North Liberty, Iowa, USA

Helio S. Sader, MD, PhD
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
ph. 319.665.3370
fax 319.665.3371
helio-sader@jmilabs.com

Amended Abstract

Background: Some Enterobacteriaceae (ENT) species are capable of producing large amounts of AmpC β-lactamase (BL) and become resistant (R) to third-generation cephalosporins. NXL104 is a novel β-lactamase (BL) inhibitor that inhibits AmpC, as well as ESBL and KPC enzymes. We evaluated the activity of ceftaroline (CPT), a novel anti-MRSA cephalosporin, combined with NXL104 (ceftaroline NXL104 [CXL104]; fixed 4 µg/mL) against clinical strains of AmpC-hyperproducing ENT.

Methods: CXL104 and 13 comparators were tested for susceptibility (S) by CLSI broth microdilution methods against 1279 ENT, including *Enterobacter* spp. (ESP; 699), *Citrobacter* spp. (CSP; 140), *Serratia* spp. (SER; 324) and indole-positive Proteae (IPP; 116), typical AmpC-producing species. The strains were consecutively collected in 2009 from 75 medical centers located in the USA (51 sites in 9 Census Regions) and Europe (EU; 24 sites in 13 nations).

Results: Overall, 15.9% (203 strains) were ceftazidime (CAZ)-R and probable AmpC-hyperproducers. CXL104 inhibited 64.6 and 96.1% of CAZ-R strains at ≤2 and ≤4 µg/mL, respectively, and 99.1% of CAZ-S at ≤2 µg/mL (Table). Isolates with CXL104 MIC at >2 µg/mL were ESP (5; 0.7%), SER (16; 4.9%) and IPP (3; 2.6%). CXL104 was highly active against CAZ-R ESP, CSP and IPP with MIC_{50}s of 0.5, 0.25, and 0.12 µg/mL, respectively, while CAZ-S and -R SER exhibited slightly higher CXL104 MICs (MIC_{50}, 0.5 and 4 µg/mL, respectively). CAZ-R ESP showed lower S rates for levofloxacin (73.9-79.3%), cefepime (81.6-88.4%), and gentamicin (64.4-78.3%), while amikacin (95.7-97.7%) and imipenem (92.0-95.7%) retained good activity against these organisms in USA and EU.

	no. of isolates(cumulative %) inhibited at CXL104 MIC (µg/mL) of:							
	≤0.06	0.12	0.25	0.5	1	2	4	8
Enterobacter spp. (699)								
Ceftazidime-S (543)	156(28.7)	222(69.6)	127(93.0)	31(98.7)	4(99.4)	2(99.8)	1(100.0)	-
Ceftazidime-R (156)	9(5.7)	21(19.2)	34(41.0)	49(72.4)	31(92.3)	8(97.4)	1(98.0)	3(98.0)[a]
Citrobacter spp. (140)								
Ceftazidime-S (116)	66(56.9)	37(88.8)	7(94.8)	4(98.2)	1(99.1)	1(100.0)	-	-
Ceftazidime-R (24)	0(0.0)	5(20.8)	11(66.7)	5(87.5)	2(95.8)	1(100.0)	-	-
Serratia spp. (324)								
Ceftazidime-S (309)	1(0.3)	4(1.6)	89(30.4)	113(67.0)	74(90.9)	20(97.4)	6(99.4)	2(100.0)
Ceftazidime-R (15)	0(0.0)	0(0.0)	0(0.0)	2(13.3)	3(33.3)	2(46.7)	2(60.0)	5(93.3)[b]
Indole-positive Proteae (116)								
Ceftazidime-S (108)	64(59.3)	19(76.9)	15(90.8)	3(93.6)	2(93.5)	4(97.2)	0(97.2)	1(100.0)
Ceftazidime-R (8)	2(25.0)	3(62.5)	1(75.0)	1(87.5)	1(100.0)	-	-	-

a. 3 metallo-β-lactamase-producing strains from Spain with CXL MIC >32 µg/mL.
b. Including 1 *S. marcescens* from France with CXL104 MIC of 16 µg/mL.

Conclusion: NXL104 can effectively lower CPT MIC values for AmpC-hyperproducer ENT. CXL104 activity against CAZ-R ENT was similar to or greater than that of imipenem.

Introduction

AmpC β-lactamases are clinically important cephalosporinases encoded on the chromosome of many Enterobacteriaceae. These enzymes mediate resistance to penicillins, cephalosporins, and β-lactamase inhibitor/β-lactam combinations, making the selection of empiric therapy difficult. In many bacteria, AmpC enzymes are inducible and can be expressed at high levels by mutation or acquisition of a genetic element supplying a promoter that increases gene expression.

Ceftaroline, the active form of the parenteral prodrug ceftaroline fosamil, is a novel broad-spectrum cephalosporin with activity against resistant Gram-positive organisms (including methicillin-resistant *Staphylococcus aureus* [MRSA] and *Streptococcus pneumoniae*) and most Enterobacteriaceae species. Like many cephalosporins, ceftaroline has limited activity against extended-spectrum β-lactamase (ESBL)- and AmpC-hyperproducing strains. NXL104 is a novel non-β-lactam β-lactamase inhibitor that displays broad-spectrum activity against both Ambler class A and class C enzymes, and a variable level of activity against class D enzymes. NXL104 has limited intrinsic antibacterial activity, but efficiently protects β-lactams from hydrolysis by a variety of organisms producing class A and C β-lactamases, including chromosomally encoded AmpC types.

The present study evaluated the activity of ceftaroline/NXL104 (ceftaroline combined with NXL104 at fixed concentration of 4 µg/mL [CXL104]) against 1279 Enterobacteriaceae isolates belonging to species that typically hyperproduce chromosomal AmpC. All isolates were collected from United States (USA) and European medical centers in 2009.

Methods

Bacterial Isolates. A total of 1279 Enterobacteriaceae isolates collected (during 2009) from 51 medical centers in all 9 USA Census Regions and in 24 hospitals from 13 European countries were analyzed. Only 1 isolate per patient from documented infections was included in this prevalence design study. Species identification was confirmed by standard biochemical tests, the Vitek System (bioMerieux; Hazelwood, Missouri, USA), or 16S rRNA sequencing, when necessary.

Antimicrobial Susceptibility Testing. All isolates were tested for antimicrobial susceptibility using the broth microdilution method (BMD) described by the CLSI (M07-A8, 2009). Cation-adjusted Mueller-Hinton broth was used in validated BMD panels. Ceftaroline was tested alone and with a fixed 4 µg/mL concentration of NXL104. Categorical interpretations were those found in CLSI; M100-S20-U and quality control (QC) were performed using *Escherichia coli* ATCC 25922 and ATCC 35218, *S. aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents.

Results

- CXL104 activity against typical AmpC-producing species, *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., and indole-positive Proteae is summarized in Table 1. Overall, CXL104 inhibited 94.6% and 96.1% of ceftazidime-resistant strains at ≤2 and ≤4 µg/mL, respectively
- CXL104 activity against ceftazidime-susceptible and -intermediate *Enterobacter* spp. from the USA and Europe (for both regions, MIC_{50}, 0.12 µg/mL and MIC_{90}, 0.25 µg/mL) was comparable to that of cefepime (for both regions, MIC_{50}, ≤0.12 µg/mL and MIC_{90}, 0.25 µg/mL; Table 2)
- Against ceftazidime-resistant (AmpC-derepressed enzyme-producing) *Enterobacter* spp., CXL104 (MIC_{50}, 0.5 µg/mL and MIC_{90}, 1 µg/mL) was at least 2-fold more active than cefepime (MIC_{50}, 1 and 2 µg/mL and MIC_{90}, 16 and >16 µg/mL for Europe and USA, respectively) and compared favorably with imipenem (MIC_{50}, 0.5 µg/mL for both regions; MIC_{90}, 1 µg/mL for Europe and 4 µg/mL for USA)
- CXL104 MIC values of >32 µg/mL were noted in 3 *Enterobacter cloacae* strains from Spain (Table 1). These isolates were also resistant to ceftazidime (MIC values of >32 µg/mL) and imipenem (MIC values of 4, 8, and >8 µg/mL), and produced the metallo-β-lactamase bla_{VIM-1}, which is not inhibited by NXL104

Table 1. Summary of CXL104 Activity Against 1279 Enterobacteriaceae Strains From USA and European Medical Centers (2009)

Organism (no. tested)	Cumulative % of strains inhibited at MIC (µg/mL):									
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
Enterobacter spp.										
Ceftazidime-S/I										
USA (360)	8.3	29.2	71.7	93.3	99.2	100.0				
Europe (183)	4.4	28.9	65.6	92.3	97.8	98.4	99.4	100.0		
Ceftazidime-R										
USA (87)	1.1	6.9	20.7	40.2	70.1	94.2	98.9	100.0		
Europe (69)	0.0	4.3	17.4	42.0	75.4	89.9	95.6	95.6	95.6	100.0[a]
Citrobacter spp.										
Ceftazidime-S/I										
USA (69)	10.1	52.2	91.3	94.2	100.0					
Europe (47)	12.7	63.8	85.1	95.7	95.7	97.9	100.0			
Ceftazidime-R										
USA (13)	0.0	0.0	23.1	69.2	92.3	100.0				
Europe (11)	0.0	0.0	18.2	63.6	81.8	90.9	100.0			
Serratia spp.										
Ceftazidime-S/I										
USA (211)	0.0	0.5	2.0	30.8	67.7	90.5	97.0	99.0	100.0	
Europe (108)	0.0	0.0	0.9	29.6	65.7	91.7	98.1	100.0		
Ceftazidime-R										
USA (9)	0.0	0.0	0.0	0.0	22.2	55.6	77.8	100.0		
Europe (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	83.3	100.0[b]
Indole-positive Proteae										
Ceftazidime-S/I										
USA (45)	24.4	60.0	73.3	84.4	86.7	88.9	95.6	100.0		
Europe (63)	25.4	58.7	77.8	92.1	95.2	96.8	98.4	98.4	100.0	
Ceftazidime-R										
USA (3)	0.0	0.0	33.3	66.7	66.7	100.0				
Europe (5)	20.0	40.0	80.0	80.0	100.0					

a. Three metallo-β-lactamase-producing strains from Spain with CXL104 MIC at >32 µg/mL.
b. One *S. marcescens* from France with CXL104 MIC of 16 µg/mL.
I = intermediate; R = resistant; S = susceptible.

- *Serratia* spp. from both regions exhibited CXL104 MIC_{50} of 0.5 µg/mL and MIC_{90} of 2 µg/mL. This activity was comparable to that of imipenem (MIC_{50}, 1 µg/mL and MIC_{90}, 2 µg/mL; Table 2). Among 8 *Serratia* spp. isolates with CXL104 MIC ≥8 µg/mL (Table 1), 6 were from a single French hospital. These strains were genetically related by pulsed-field gel electrophoresis and carried only bla_{TEM-1} among several β-lactamase genes screened (data not shown).

Table 2. Activity of CXL104 and Comparator Antimicrobial Agents When Tested Against Enterobacteriaceae Isolates Belonging to Species That Produce Chromosomal AmpC Enzymes

Organism (no. tested)/ Antimicrobial Agent	USA (no. of strains)				Europe (no. of strains)			
	MIC		%Susc.		MIC		% Susc.	
	50%	90%	CLSI[a]	EUCAST[a]	50%	90%	CLSI[a]	EUCAST[a]
Enterobacter spp.		(447)				(252)		
CXL104	0.12	0.5	-	-	0.12	0.5	-	-
Ceftaroline	0.25	>32	-	-	0.25	>32	-	-
Ceftriaxone	≤0.25	>32	76.1	76.1	≤0.25	>32	64.7	64.7
Cefepime	≤0.12	2	96.2	86.8	≤0.12	2	96.8	87.3
Imipenem	0.5	2	98.4	98	0.5	1	98.4	97.6
Amikacin	1	2	99.6	98.9	1	2	98.8	97.2
Gentamicin	≤2	≤2	98	93.1	≤2	≤2	93.7	92.5
Levofloxacin	≤0.5	1	94.4	92.2	≤0.5	4	89.7	88.1
Ceftazidime-S/I *Enterobacter* spp.		(360)				(183)		
CXL104	0.12	0.25	-	-	0.12	0.25	-	-
Ceftaroline	0.25	1	-	-	0.25	2	-	-
Ceftriaxone	≤0.25	0.5	94.4	94.4	≤0.25	2	89.1	89.1
Cefepime	≤0.12	0.25	99.7	99.7	≤0.12	0.25	100	100
Imipenem	0.5	1	100	100	0.5	1	99.5	98.9
Amikacin	1	2	100	100	1	2	100	100
Gentamicin	≤2	≤2	99.4	98.9	≤2	≤2	99.5	98.9
Levofloxacin	≤0.5	≤0.5	98.1	97.2	≤0.5	≤0.5	95.6	95.6
Ceftazidime-R *Enterobacter* spp.		(87)				(69)		
CXL104	0.5	1	-	-	0.5	2	-	-
Ceftaroline	>32	>32	-	-	>32	>32	-	-
Ceftriaxone	>32	>32	0	0	32	>32	0	0
Cefepime	2	>16	81.6	33.3	1	16	88.4	53.6
Imipenem	0.5	4	92	89.7	0.5	1	95.7	94.2
Amikacin	1	8	97.7	94.3	1	16	95.7	89.9
Gentamicin	≤2	>8	64.4	63.2	≤2	>8	78.3	75.4
Levofloxacin	≤0.5	>4	79.3	71.3	≤0.5	>4	73.9	89.9
Citrobacter spp.		(82)				(58)		
CXL104	0.12	0.25	-	-	0.06	0.5	-	-
Ceftaroline	0.25	>32	-	-	0.25	>32	-	-
Ceftriaxone	≤0.25	32	78	78	≤0.25	32	77.6	77.6
Cefepime	≤0.12	1	100	95.1	≤0.12	1	94.8	91.4
Imipenem	0.5	1	100	100	0.5	1	100	100
Amikacin	1	2	100	97.6	1	4	100	98.3
Gentamicin	≤2	≤2	98.8	96.3	≤2	≤2	94.8	93.1
Levofloxacin	≤0.5	1	95.1	91.5	≤0.5	1	93.1	93.1
Ceftazidime-S/I *Citrobacter* spp.		(69)				(47)		
CXL104	0.06	0.12	-	-	0.06	0.25	-	-
Ceftaroline	0.12	1	-	-	0.25	0.5	-	-
Ceftriaxone	≤0.25	0.5	92.8	92.8	≤0.25	≤0.25	95.7	95.7
Cefepime	≤0.12	0.25	100	97.1	≤0.12	≤0.12	100	100
Imipenem	0.5	1	100	100	0.5	1	100	100
Amikacin	1	2	100	97.1	1	2	100	100
Gentamicin	≤2	≤2	100	98.6	≤2	≤2	97.9	97.9
Levofloxacin	≤0.5	≤0.5	98.6	97.1	≤0.5	≤0.5	97.9	97.9
Ceftazidime-R *Citrobacter* spp.		(13)				(11)		
CXL104	0.25	0.5	-	-	0.25	1	-	-
Ceftaroline	>32	>32	-	-	>32	>32	-	-
Ceftriaxone	32	>32	0	0	32	>32	0	0
Cefepime	1	2	100	84.6	1	>16	72.7	54.5
Imipenem	0.5	1	100	100	1	2	100	100
Amikacin	1	2	100	100	2	8	100	90.9
Gentamicin	≤2	4	92.3	84.6	≤2	>8	81.8	72.7
Levofloxacin	≤0.5	4	76.9	61.5	≤0.5	>4	72.7	72.7

Organism (no. tested)/ Antimicrobial Agent	USA (no. of strains)				Europe (no. of strains)			
	MIC		%Susc.		MIC		% Susc.	
	50%	90%	CLSI[a]	EUCAST[a]	50%	90%	CLSI[a]	EUCAST[a]
Serratia spp.		(210)				(114)		
CXL104	0.5	2	-	-	0.5	2	-	-
Ceftaroline	1	8	-	-	1	>32	-	-
Ceftriaxone	≤0.25	2	88.1	88.1	≤0.25	8	76.3	76.3
Cefepime	≤0.12	0.5	99	94.8	≤0.12	0.5	100	98.2
Imipenem	1	2	99	97.1	1	2	100	98.2
Amikacin	2	4	100	100	2	4	100	98.2
Gentamicin	≤2	≤2	94.8	93.8	≤2	≤2	93.9	93
Levofloxacin	≤0.5	1	95.7	90.5	≤0.5	2	90.4	84.2
Ceftazidime-S/I *Serratia* spp.		(201)				(108)		
CXL104	0.5	1	-	-	0.5	1	-	-
Ceftaroline	1	4	-	-	1	32	-	-
Ceftriaxone	≤0.25	1	92	92	≤0.25	8	80.6	80.6
Cefepime	≤0.12	0.25	100	98.5	≤0.12	0.5	100	98.1
Imipenem	1	2	86.1	98	1	2	83.3	98.1
Amikacin	2	4	100	100	2	4	100	99.1
Gentamicin	≤2	≤2	99	98	≤2	≤2	99.1	98.1
Levofloxacin	≤0.5	1	97.5	92.5	≤0.5	2	93.5	88.9
Ceftazidime-R *Serratia* spp.		(9)				(6)		
CXL104	1	-	-	-	8	-	-	-
Ceftaroline	32	-	-	-	>32	-	-	-
Ceftriaxone	32	-	0	0	8	-	0	0
Cefepime	4	-	77.8	11.1	0.5	-	100	100
Imipenem	1	-	55.6	77.8	0.5	-	100	100
Amikacin	1	-	100	100	4	-	100	83.3
Gentamicin	>8	-	0	0	>8	-	0	0
Levofloxacin	2	-	55.6	44.4	4	-	33.3	0
Indole-positive *Proteus* spp. (IPP)		(48)[b]				(68)[c]		
CXL104	0.06	2	-	-	0.06	0.25	-	-
Ceftaroline	0.12	>32	-	-	0.5	>32	-	-
Ceftriaxone	≤0.25	2	85.4	85.4	≤0.25	4	80.9	80.9
Cefepime	≤0.12	0.25	100	97.9	≤0.12	≤0.12	100	98.5
Imipenem	2	4	100	72.9	2	4	98.5	58.8
Amikacin	2	4	100	100	2	4	100	100
Gentamicin	≤2	>8	75	64.6	≤2	4	91.2	88.2
Levofloxacin	≤0.5	>4	68.8	66.7	≤0.5	>4	85.3	82.4
Ceftazidime-S/I IPP		(45)				(63)		
CXL104	0.6	2	-	-	0.06	0.25	-	-
Ceftaroline	0.12	16	-	-	0.25	>32	-	-
Ceftriaxone	≤0.25	1	91.1	91.1	≤0.25	2	87.3	87.3
Cefepime	≤0.12	0.25	100	100	≤0.12	≤0.12	100	98.4
Imipenem	2	4	24.4	71.1	2	4	15.9	57.1
Amikacin	>16	>16	6.7	-	2	4	100	100
Gentamicin	≤2	>8	75.6	64.4	≤2	4	92.1	88.9
Levofloxacin	≤0.5	>4	73.3	71.1	≤0.5	4	87.3	84.1
Ceftazidime-R IPP		(3)[d]				(5)[e]		
CXL104	0.25	-	-	-	0.12	-	-	-
Ceftaroline	>32	-	-	-	>32	-	-	-
Ceftriaxone	4	-	0	0	4	-	0	0
Cefepime	≤0.12	-	100	66.7	≤0.12	-	100	100
Imipenem	2	-	0	100	2	-	0	80
Amikacin	4	-	100	100	4	-	100	100
Gentamicin	≤2	-	66.7	66.7	≤2	-	80	80
Levofloxacin	>4	-	0	0	1	-	60	60

a. Criteria as published by the CLSI [2010] and EUCAST [2010]. Breakpoints for ceftaroline and CXL104 have not been defined.
b. Includes: *Morganella morganii* (31 strains), *Proteus vulgaris* (5 strains), *Providencia rettgeri* (2 strains), *Providencia stuartii* (9 strains), and unspeciated *Morganella* (1 strain).
c. *Morganella morganii* (49 strains), *Proteus vulgaris* (11 strains), *Providencia stuartii* (3 strains), unspeciated *Proteus* (2 strains), and unspeciated *Providencia* (3 strains).
d. *Morganella morganii* (3 strains).
e. *Morganella morganii* (5 strains).
EUCAST = European Committee on Antimicrobial Susceptibility Testing; I = intermediate; R = resistant; S = susceptible.

Conclusions

- CXL104 exhibits potent in vitro activity against Enterobacteriaceae species that hyperproduce chromosomal AmpC, including ceftazidime-resistant strains that tend to have high levels of expression of the intrinsic cephalosporinase
- CXL104 appears to be a novel option for treatment of these infections, with in vitro activity has been shown to be comparable to that of the carbapenems and fourth-generation cephalosporins.

References

1. Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: eighth edition.* Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute (2010). *M100-S20. Performance standards for antimicrobial susceptibility testing: 20th informational supplement*. Wayne, PA: CLSI.
3. Endimiani A, Choudhary Y, Bonomo RA (2009). In vitro activity of NXL104 in combination with beta-lactams against *Klebsiella pneumoniae* isolates producing KPC carbapenemases. *Antimicrob Agents Chemother* 53: 3599-3601.
4. Jacobs MR, Good CE, Windau AR, Bajaksouzian S, Biek D, Critchley IA, Sader HS, Jones RN (2010). Activity of ceftaroline against recent emerging serotypes of *Streptococcus pneumoniae* in the United States. *Antimicrob Agents Chemother* 54: 2716-2719.
5. Jacoby GA (2009). AmpC beta-lactamases. *Clin Microbiol Rev* 22: 161-182.
6. Jones RN (1998). Important and emerging beta-lactamase-mediated resistances in hospital-based pathogens: The Amp C enzymes. *Diagn Microbiol Infect Dis* 31: 461-466.
7. Mushtaq S, Warner M, Williams G, Critchley I, Livermore DM (2010). Activity of chequerboard combinations of ceftaroline and NXL104 versus beta-lactamase-producing Enterobacteriaceae. *J Antimicrob Chemother* 65: 1428-1432.
8. Zhanel GG, Sniezek G, Schweizer F, Zelenitsky S, Lagace-Wiens PR, Rubinstein E, Gin AS, Hoban DJ, Karlowsky JA (2009). Ceftaroline: A novel broad-spectrum cephalosporin with activity against meticillin-resistant *Staphylococcus aureus*. *Drugs* 69: 809-831.

Acknowledgment

Supported by Forest Laboratories, Inc.